C2

69. (new) A process for the production of a mature recombinant protein in the culture medium of an eukaryotic cell line which has been genetically transfected with a cDNA sequence encoding for a protein precursor selected from the group consisting of: preproenzymes, zymogens, matrix metallo proteases, comprising incubating said cell line in the cell culture medium for a time of at least 24 hours, to which has been added wherein with an alkanoic acid selected from the group consisting of butyric acid, sodium butyrate, sodium propionate, magnesium butyrate, tributyrin and phenyl-butyrate, their derivatives or salts thereof.

70. (new) A process according to claim 2 wherein said protein precursor is pre-prourokinase or prourokinase and said mature recombinant protein is two chain-urokinase.

71. (new) A process according to claim 70 wherein said two chain-urokinase is High Molecular Weight two chain-urokinase and Low Molecular Weight two chain-urokinase and the eukaryotic cell line is genetically transfected with a cDNA sequence encoding for the human Pre-prourokinase, comprising:

incubating said cell line for a time of at least 24 hours, in a cell culture medium to which has been added an alkanoic acid selected from the group consisting of: butyric acid, sodium butyrate, sodium propionate, magnesium butyrate, tributyrin and phenyl-butyrate, their derivatives or salts thereof;

recovering a cell culture supernatant;

performing ion exchange chromatography on the cell culture supernatant wherein Low Molecular Weight two chain-urokinase is released by addition of a buffer solution with pH from 5.5 to 6.5, said solution further comprising a monovalent ion in concentration from 200 mM to 300 mM and wherein the High Molecular Weight two chain-urokinase is released by addition of a buffer solution with pH from 6 to 7.5, said solution further comprising a monovalent ion in concentration of at least 400 mM and optionally further purifying Low Molecular Weight and High Molecular Weight two chain-urokinase by benzamidine

chromatography.

72. (new) A process according to claim 71 wherein the concentration of said alkanoic acids is comprised from 0.1 to 20 mM.

73. (new) A process according to claim 71 wherein said eukaryotic cell line is a mammalian cell line selected from the group consisting of: HEK-293, CV-1, COS, BSC-1, MDCK, A-431, CHO, BHK, CHO-Messi.

C2 cont.

74. (new) A process according to claim 73 wherein said eukaryotic cell line is selected from CHO and CHO-Messi.

75. (new) A process according to claim 71 wherein in step a) said temperature is in the range from 30°C to 37°C.

76. (new) A process according to claim 75 wherein said temperature is in the range from 33°C to 35°C.

77. (new) A process according to claim 71 wherein said time of incubation in step a) is in the range from 48 to 200 hours

78. (new) A process according to claim 77 wherein said time of incubation in step a) is in the range from 72 to 150 hours.

79. (new) A process according to claim 71 wherein said cell culture medium is serum-free.

80. (new) A process according to claim 71 wherein the supernatant recovered in step b) is acidified to a pH ranging from 5 to 5.8, a non-ionic detergent is added and the supernatant is filtered.

REMARKS

The Examiner is thanked for examining claims 1 and 40-55 on the merits in view of the prior Amendment of June 3, 2002.